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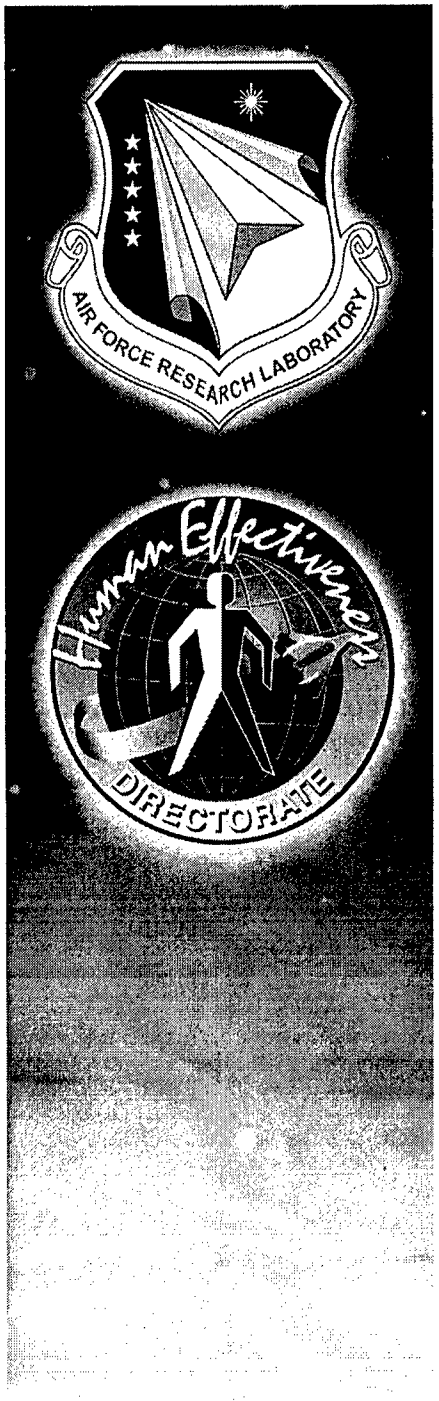

Air Force Research Laboratory

Examination of HFE C282Y/H63D Heterozygotes as a Potential Human Modeling System for Low Level Liver Damage

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14. ABSTRACT

Individuals heterozygous for hemochromatosis gene (HFE) mutations have been shown to demonstrate mild systemic iron-loading. In some studies, mildly elevated liver enzymes are noted. More sensitive testing methodologies are needed to assist in determining which heterozygous individuals may be at risk for future deleterious effects. Alpha Glutathione-S-Transferase (AGST) has been used to determine sub-clinical liver dysfunction and damage. However, AGST has not been used to examine the potential damage caused by mild iron loading. Whole blood/serum samples from individuals 20-50 years of age were collected from the Wright Patterson AFB clinical laboratory with only age and sex indicated. Genomic DNA was isolated from anticolagulated whole blood using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences). In two reactions, Exon 2 and Exon 4 of the HFE1 gene were amplified using primer sets HH63A/HH63B and HH1/HH5, respectively, in a standard PCR reaction (Accuprime PCR Kit, Invitrogen). The PCR products were digested either with MboI (Exon 2 containing the C186G mutation) or RsaI (Exon 4 containing the G845A mutation). The resultant restriction fragments were analyzed with matched controls on a 2% TBE agarose gel. Three groups of samples were identified (C282Y heterozygotes, H63D heterozygotes, and homozygous normal controls). Serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, iron status, and AGST were analyzed on each sample. Samples from individuals possessing heterozygous C282Y mutation did not demonstrate a statistically significant elevation in AGST or other liver enzymes when compared to samples with no mutation. Similar results were found in individuals heterozygous for the H63D mutation. These results demonstrate that AGST activity may not be a good indicator for sub-clinical liver damage caused by increased loading of hepatic iron. Thus, the data does not support the use of human HFE C282Y/H63D heterzygtote samples in sub-clinical human liver dysfunction modeling.

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EXAMINATION OF HFE C282Y/H63D HETEROZYGOTES AS A POTENTIAL HUMAN MODELING SYSTEM FOR LOW LEVEL LIVER DAMAGE

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Abstract

Individuals heterozygous for hemochromatosis gene (HFE) mutations have been shown to demonstrate mild systemic iron-loading. In some studies, mildly elevated liver enzymes are noted. More sensitive testing methodologies are needed to assist in determining which heterozygous individuals may be at risk for future deleterious effects. Alpha Glutathione-S-Transferase (AGST) has been used to determine sub-clinical liver dysfunction and damage. However, AGST has not been used to examine the potential damage caused by mild iron loading. Whole blood/serum samples from individuals 20-50 years of age were collected from the Wright Patterson AFB clinical laboratory with only age and sex indicated. Genomic DNA was isolated from anticolagulated whole blood using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences). In two reactions, Exon 2 and Exon 4 of the HFE1 gene were amplified using primer sets HH63A/HH63B and HH1/HH5, respectively, in a standard PCR reaction (Accuprime PCR Kit, Invitrogen). The PCR products were digested either with MboI (Exon 2 containing the C186G mutation) or RsaI (Exon 4 containing the G845A mutation). The resultant restriction fragments were analyzed with matched controls on a 2% TBE agarose gel. Three groups of samples were identified (C282Y heterozygotes, H63D heterozygotes, and homozygous normal controls). Serum alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, iron status, and AGST were analyzed on each sample. Samples from individuals possessing heterozygous C282Y mutation did not demonstrate a statistically significant elevation in AGST or other liver enzymes when compared to samples with no mutation. Similar results were found in individuals heterozygous for the H63D mutation. These results demonstrate that AGST activity may not be a good indicator for sub-clinical liver damage caused by increased loading of hepatic iron. Thus, the data does not support the use of human HFE C282Y/H63D heterzygtote samples in sub-clinical human liver dysfunction modeling.

Introduction

Hereditary hemochromatosis (HHC) is an autosomal recessive disorder that results in increased iron absorption in affected individuals.(1) In 1996, the HFE gene was identified,(2) and mutations of this gene have been called the single most common gene disorder in Caucasian populations.(3) Multiple mutations of this gene have been identified and two major variants C282Y and H63D of the HFE gene have been

associated with clinical conditions.(1) Over time, increased iron absorption through the gastrointestinal tract in individuals affected with hemochromatosis (HHC) and this chronic iron loading state has been correlated with increased occurrence of a number of pathologic conditions including liver cancer and arthritis as well as lower states of well being with increase in fatigue.(4) The prevalence of C28Y homozygotes varies with ethnicity mixture of the population. Non-Hispanic Caucasians (0.44 percent) have the highest C28Y homozygotic prevalence(5). Although varying by population, the overall allele frequencies of the C282Y and H63D mutations have been reported penetrate at approximately 8 and 15 percent, respectively(6). Carriers of the C282Y mutations appear to have a stronger genetic correlation for prognosis dominated with ensuing pathology and age related clinical impact than H63D mutations.(1) Individuals heterozygous for either mutation normally have no clinical symptomology (1). Heterozygous individuals, especially those with a C282Y mutation, often have mild elevations of liver enzymes (i.e. alanine aminotransferase).(7) These mild elevations, along with evidence of synergistic relationships with dietary/environmental factors (i.e. clinical symptoms for C282Y mutation with alcohol intake), suggest that individuals heterozygous with HHC associated mutations may experience minimally intermittent or chronic mild liver damage.(7) Identification of individuals with mild HHC associated liver damage would provide a tremendous advantage to current treatments/diagnostic tools. The definitive diagnosis of liver associated damage attributed to HHC is biopsy.(1) An alternative is suggested due to the complications associated with biopsy. Alternatively, if standard iron levels (transferrin saturation and ferritin) are elevated, individuals may obtain therapeutic phlebotomies to lower iron burden.(1) These procedures, while certainly less invasive than biopsy, are cumbersome. Therefore, identification of the extent of the subpopulation exhibiting iron burden and liver damage at a "low level" or under normal dietary conditions is a worthy goal for screening this very large C282Y heterozygote population. There are many biomarkers currently available to study. One such biomarker is Alpha-glutathione-S-transferase(AGST).(8) In the liver, AGST is enzyme located in the hepatocytes and has been shown in may acute and some chronic states to be elevated before traditional liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), and alkaline phosphatase(AP)) have been elevated.(9-13) To date, AGST has not been used to investigate potential low level liver damage due to HFE mutations.

Objective

The objective of this study was to evaluate serum AGST level as a possible indicator for low level human liver damage caused by mutations in the HFE gene.

Materials and Methods

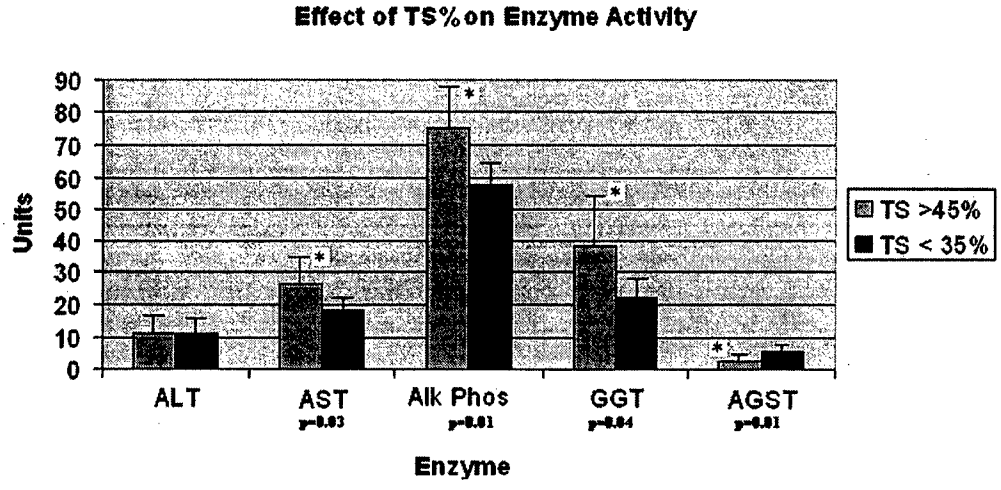
Whole blood/serum samples from individuals 20-50 years of age were collected from the Wright Patterson AFB clinical laboratory. Only age and sex were recorded. Genomic DNA was isolated from anticolagulated whole blood using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences). In two reactions, Exon 2 and Exon 4 of the HFE1 gene were amplified using primer sets HH63A/HH63B and HH1/HH5,

respectively, in a standard PCR reaction (Accuprime PCR Kit, Invitrogen). The PCR products were digested either with MboI (Exon 2 containing the C186G mutation) or RsaI (Exon 4 containing the G845A mutation). The resultant restriction fragments were analyzed with controls on a 2% TBE agarose gel. Three groups of samples were identified (C282Y heterozygotes, H63D heterozygotes, and homozygous normal controls). ALT, AST, GGT. AP, total iron and transferrin saturation (TS) were analyzed using standard procedures (Cobas Mira, Roche Diagnostics). AGST (Biotrin) was analyzed for each sample using microtiter plate antibody assay. Statistical analysis was performed using the Number Cruncher Statistical System. This study was approved by the 88th Medical Group IRB.

Results

	Control (n=24)	C282y +/- (n=10)	H63D +/- (n=7)	Compound (n=6)
ALT (U/L)	14.1	8.5	14.4	5.5
AST (U/L)	26.3	19.8	21.7	16.5
Alk Phos (U/L)	72.35	59.5	58.6	51.6
GGT (U/L)	38.2	19.3	20.6	20.6
AGST (U/L)	5.62	3.93	3.99	1.99
Iron (ug/dL)	175	113	155	129
TS%	38.3	35	47.7	47.8

	Age <39 (n=26)	Age >39 (n=26)	Male (n=14)	Female (n=39)
ALT (U/L)	11.2	12	17	9.5
AST (U/L)	20.4	24.7	24.3	22.1
Alk Phos (U/L)	57	74	62	66
GGT (U/L)	20.5	37.2	29.7	28.4
AGST (U/L)	5.4	4.5	6.05	4.62
Iron (ug/dL)	117	181	140	154
TS%	36	41	41	38
Age	28	45	34	38



Conclusion

Samples from individuals heterozygous for the C282Y mutation did not demonstrate a statistically significant elevation in AGST or other liver enzymes when compared to samples with no mutation. Instead, an average lower level value was determined in these individuals. No elevation was found in individuals heterozygous for the H63D mutation and in compound heterogygotes. These results demonstrate that elevated serum levels of AGST protein may not be a good indicator for sub-clinical liver damage caused by manifestations of mutations in the HFE gene. Surprisingly, individuals in this sampling of middle age individuals with mutations in the HFE gene did not have elevated iron status results. Examination of samples with elevated transferrin saturation percentage did show elevated liver enzyme activity with the exception being ALT and AGST, which showed a statistically significant decrease. These results do not support the initial hypothesis that AGST would be elevated in serum, could be used as detection of the condition, and could to demonstrate low level hepatic damage in individuals with HFE C282Y/H63D mutations. However, these data did show that lower levels of AGST were found in affected individuals. This counterintuitive finding may suggest a compensatory regulation of AGST or a lower hepatic AGST enzyme content in these individuals. Further investigation is merited, since increases in AGST levels are presently assumed as an event (an acute) hepatic event, where this appears not to be the clear case for chronic liver iron handling/stressor conditions.

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